

channel function using planar lipid bilayers (PLBs) and integrity using analytical size exclusion chromatography (A-SEC) in the detergent-solubilized state. We also examined receptor mobility on the lipidic cubic phase (LCP) by measuring nAChR mobile fraction and diffusion coefficient through fluorescence recovery after photobleaching (FRAP) experiments using lipid-analog and non-lipid-analog detergents. Our results show that it is possible to isolate stable and functional nAChRs using lipid-analog detergents, with characteristic ion channel currents in PLBs and minimal aggregation as observed in A-SEC. Furthermore, fractional mobility and diffusion coefficient values observed in FRAP experiments were similar to the values observed for these parameters in the recently LCP-crystallized $\beta 2$ -adrenergic receptor. The overall results show that phospholipid-analog detergents with 16 carbon acyl-chains support nAChR integrity, functionality, and LCP mobility.

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X-Ray Structures of General Anaesthetics Bound to a Pentameric Ligand-Gated Ion Channel

Hugues Nury¹, Catherine Van Renterghem¹, Yun Weng², Alfonso Tran², Marc Baaden³, Virginie Dufresne¹, Jean-Pierre Changeux¹, Jim Sonner², Marc Delarue¹, Pierre-Jean Corringer¹.

¹Institut Pasteur, Paris, France, ²UCSD, San Francisco, CA, USA,

³CNRS, Paris, France.

General anaesthetics have enjoyed long and widespread use but their molecular mechanism of action remains poorly understood. There is good evidence that their principal targets are pentameric ligand-gated ion channels (pLGICs) such as inhibitory GABAA (γ -aminobutyric acid) receptors and excitatory nicotinic acetylcholine receptors, which are respectively potentiated and inhibited by general anaesthetics. The bacterial homologue from *Gloeobacter violaceus* (GLIC), whose X-ray structure was recently solved, is also sensitive to clinical concentrations of general anaesthetics. Here we describe the crystal structures of the complexes propofol/GLIC and desflurane/GLIC. These reveal a common general-anaesthetic binding site, which pre-exists in the apo-structure in the upper part of the transmembrane domain of each protomer. Both molecules establish van der Waals interactions with the protein; propofol binds at the entrance of the cavity whereas the smaller, more flexible, desflurane binds deeper inside. Mutations of some amino acids lining the binding site profoundly alter the ionic response of GLIC to protons, and affect its general-anaesthetic pharmacology. Molecular dynamics simulations, performed on the wild type and two GLIC mutants, highlight differences in mobility of propofol in its binding site and help to explain these effects. These data provide a novel structural framework for the design of general anaesthetics and of allosteric modulators of brain pLGICs.

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New Estimates for the 'Allosteric' Constant of Neuromuscular Acetylcholine Receptor-Channels

Tapan K. Nayak, Prasad Purohit, Anthony Auerbach.

SUNY @ Buffalo, Buffalo, NY, USA.

Nicotinic acetylcholine receptors (AChRs) open rarely in the absence of agonists but many different mutations substantially increase the unliganded gating equilibrium constant (the 'allosteric' constant, E_0). In the adult mouse neuromuscular AChR a single mutation, 'Special character' A96H (in both 'Special character' subunits), increased E_0 by $>10^5$ -fold and caused spontaneous openings to occur in clusters arising from individual receptors. We measured E_0 for three different sets of mutant combinations and by extrapolation estimated E_0 for wild type AChRs. The estimates were $E_0^{wt}=6.0 \times 10^{-7}$ and 7.4×10^{-7} in adult-type AChRs and 4.3×10^{-7} in fetal-type AChRs (-100 mV, 23°C). The adult value is in excellent agreement with one obtained previously by using a completely different method (6.6×10^{-7} , from monoliganded gating). We found that E_0^{wt} decreased e-fold with a ~ 58 mV depolarization, and that Na^+ and K^+ in the extracellular solution had no effect on E_0 . However, Cs^+ acted as a very weak 'partial' agonist. The results are discussed with regard to the energetics of receptor activation and the competitive antagonism of ions with regard to agonist binding.

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Identification of Amino Acid Residues that Prevent Efficient Binding of 4/7 α -Conotoxins to Nicotinic $\alpha 4\beta 2$ Receptor Subtypes

Mirko Beissner¹, Sébastien Dutertre², Rudolf Schemm³, Timm Danker⁴, Annett Sporning⁵, Annette Nickes⁵.

¹MPI for Brain Research, Frankfurt, Germany, ²Institute for Molecular Biosciences, Brisbane, Australia, ³MPI for Biophysical Chemistry, Göttingen, Germany, ⁴NMI Technologie Transfer GmbH, Reutlingen, Germany, ⁵MPI for Experimental Medicine, Göttingen, Germany.

α -Conotoxins are nAChR antagonists with high subtype selectivity. Several potent $\alpha 3\beta 2$ nAChR-selective α -conotoxins have been identified. However, most characterized α -conotoxins show no or only weak affinity for the $\alpha 4\beta 2$ nAChR subtype. In this study, we constructed homology models of $\alpha 3\beta 2$ and $\alpha 4\beta 2$ ligand binding domains and substituted selected amino acid residues in the $\alpha 4$ subunit by the respective residues in the $\alpha 3$ subunit to identify the determinants of subtype selectivity. Characterization of these mutants by two-electrode voltage-clamp analysis identified two mutants, $\alpha 4\text{R185I}$ and $\alpha 4\text{P195Q}$, that exhibited increased affinity for several α -conotoxins. Replacement of $\alpha 4\text{R185}$ by alanine or glutamate but not by lysine also increased α -conotoxin affinity, indicating that a positive charge in this position interferes with α -conotoxin binding. Docking studies and molecular dynamic simulations supported our findings and further demonstrated that residues R185 and P195 inhibit a close contact of the C-loop with α -conotoxins and thus prevent high affinity binding.

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On the Complexity of the Kinetics of Unliganded Gating in Neuromuscular Acetylcholine Receptor-Channels

Prasad G. Purohit, Anthony Auerbach.

Suny Buffalo, Buffalo, NY, USA.

The magnitude of an agonist-induced response depends on both the intrinsic tendency of the unliganded receptor to open and the amount of agonist binding energy realized in the channel-opening process. It is easy to make mouse neuromuscular acetylcholine receptors (AChRs) open in the absence of agonist by making a small number of mutations (cell attached, HEK cells, -100 mV). However, the single-channel, unliganded gating kinetics for an individual AChR is complex and show multiple closed and open components (3C, 2O). We have found that many perturbations (in the vicinity of the transmitter binding site) increase or decrease this complexity. Mutations at positions $\alpha 96$, $\alpha 153$, $\alpha 189$, or $\epsilon 121(\delta 123)$ increase the probability of longer openings, as does the presence of extracellular Cs^+ . Agonists and most mutations at positions $\alpha 93$, $\alpha 147$, $\alpha 149$, $\alpha 190$ or $\alpha 198$ greatly reduce these events. We compared log-likelihood values for all possible 3C-2O kinetic schemes (without cycles) and found that four of these consistently produced equivalently-high scores. However, only some were able to reproduce the rapid rising phase of the synaptic response apparent under normal physiological conditions. Although we as yet do not have an understanding of the structural basis for the kinetic complexity, the results suggest that without ligands there is a conformational flexibility at the binding site that is absent with ligands. The malleability of unliganded gating kinetics further suggests that many binding site residues can influence this flexibility. Supported by NS-23513 and NS-64969.

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Effects of Mutations of Proline Residues Located in the Complementary Subunit of the Acetylcholine Receptor Transmitter Binding Site

Shaweta Gupta, Prasad Purohit, Anthony Auerbach.

University at Buffalo, Buffalo, NY, USA.

Nicotinic acetylcholine receptor-channels (AChRs) are allosteric proteins that isomerize between a non-conducting/low-affinity and a conducting/high-affinity conformation. Each neuromuscular AChR has two transmitter binding sites located at the interface between adjacent subunits (α_e or α_s). We studied the gating properties of AChRs with mutations at the vicinal prolines located at these interfaces. In both cases, mutations of the N-terminal residue had little effect but those of the C-terminal residue ($\epsilon 121$ or $\delta 123$) decreased substantially the open channel probability ($500 \mu\text{M}$ ACh). We estimated the unliganded (E_0) and diliganded (E_2) gating equilibrium constants for mutations of these two prolines from single-channel currents (30 mM ACh, $+100 \text{ mV}$). All of the tested substitutions decreased both gating constants. The slope of the rate-equilibrium plot (Φ) for $\epsilon 121/\delta 123$ was ~ 0.96 (diliganded) and ~ 0.70 (unliganded). Some of the mutations caused a large decrease in the closed/open equilibrium dissociation constant ratio for ACh (λ^{ACh}) while others had more modest effects. Probing the effects of $\epsilon 121$ and $\delta 123$ mutations should allow an estimation of the binding and gating properties of each of the two transmitter binding sites, separately.

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Introducing Kappa: An Integrated, 'Catch-And-Hold' Reaction Coordinate that Triggers AChR Gating

Snehal V. Jadey, Anthony Auerbach.

State University at New York at Buffalo, Buffalo, NY, USA.

For many years drug action on membrane receptors has been conceptualized as occurring in two distinct steps, initially termed 'affinity' and 'intrinsic activity'. In ligand-gated ion channels these stages of activation are called 'binding' and 'gating'. Single-channel kinetic analysis of adult-type mouse neuromuscular